

HYDROCYANIC ACID IN PLANTS.

PART II. ITS OCCURRENCE IN THE GRASSES OF NEW SOUTH WALES.

BY JAMES M. PETRIE, D.Sc., F.I.C., LINNEAN MACLEAY FELLOW
OF THE SOCIETY IN BIOCHEMISTRY.*(From the Physiological Laboratory of the University of Sydney.)*

The systematic examination of Grasses for cyanogen compounds was the direct outcome of tests made to ascertain the cause of the sudden fatalities among stock, which took place in this State about two years ago. The sheep apparently had eaten nothing besides grass, and this grass when tested was found to contain a cyanogenetic glucoside and the corresponding enzyme.

It was conceived, that at least some of the frequent deaths from unknown causes, and which are often attributed to supposed poisonous plants, might possibly be due to such grasses.

Reference to the literature on this subject shows that hydrocyanic acid in grasses, was first discovered by Jorissen, in 1884, in *Poa aquatica* Linn., and this was followed by its detection in the sorghums, in 1902 (Dunstan and Henry). Up to the present, all the cyanophoric grasses recorded are included in about 14 genera, and are given in Table i.

Some of these exotic grasses have been naturalised in this country, and among them *Briza minor*, *Lamarckia aurea*, and *Poa pratensis*, are recorded by Couperot, as yielding hydrocyanic acid, when tested by him. (Journ. Pharm. Chim., 1908, 28, 542).

These three grasses growing in this State, have been examined at various seasons, and have never given positive results, neither did they contain any trace of an enzyme capable of decomposing amygdalin.

With regard to this peculiarity, we may compare the results of the Armstrongs and Horton (Proc. Roy. Soc. Lond., B.86, 1913, 265), with *Lotus corniculatus* growing in different countries. In apparently identical plants, they found that most contained both a cyanophoric glucoside and enzyme, but that in certain countries, the plants were acyanophoric. Of the latter, some were rich in enzyme, others contained only a trace. They state in explanation, that the presence of the two correlated factors mentioned is not sufficient, and that a third factor is necessary, probably one influencing concentration. It would appear then, that the conditions of concentration are unsuitable in some instances, such as in our three grasses.

TABLE i.

CYANOGENETIC GRASSES PREVIOUSLY KNOWN.

- Bambusa arundinacea* Roxb., 1911,* cultivated in N.S.W.
Briza minor Linn., 1908, naturalised in N.S.W.
Catabrosia aquatica Beauv., 1908.
Cortaderia argentea Stapf, 1906, cultivated in N.S.W. *C. conspicua*, *C. kermesiana*, 1906.
Elymus spp.
Festuca poa Kunth, 1908.
Holcus lanatus Linn., 1908, naturalised.
Lamarckia aurea Mœnch., 1908, naturalised.
Melica altissima, *M. ciliata*, *M. nutans*, *M. uniflora*.
Panicum maximum, *P. muticum*, 1903, introduced, *P. junceum*.
Poa aquatica Linn., 1884; *P. pratensis* Linn., 1908, naturalised.
Sorghum vulgare Pers., 1902, introduced; *S. halepense* Pers., native; *S. saccharatum*, *S. tartaricum*, 1903, introduced; *S. nigrum*.
Stipa capillata, *S. gigantea*, *S. hystericina*, *S. leptostachya*, *S. Lessingiana*, *S. tortilis*, 1906.
Zea Mays, 1903, naturalised.

We have now to add to the above list of cyanogenetic grasses the names of 17 more species, which are found in New South Wales,

* The dates refer to record of hydrocyanic acid.

and which are here recorded for the first time as containing a cyanogenetic glucoside and the correlated enzyme.

TABLE ii.

CYANOGENETIC GRASSES OF NEW SOUTH WALES.

- Andropogon gryllus* Linn., N.S.Wales native grass.
halepensis Sibth., var. *mutica*, N.S.W. native.
sorghum (L.) Brot., vars., introduced.
intermedius R.Br., N.S.W. native.
ischæmum Linn., introduced from N. America.
micranthus Kunth, N.S.W. native (scented grass).
Anisopogon avenaceus R.Br., N.S.W. native.
Bouteloua oligostachya Torr., introduced from Mexico.
Chloris petrcæ Sw., introduced.
polydactyla Sw., introduced from S. Amer.
truncata R.Br., N.S.W. native (star grass).
ventricosa R.Br., N.S.W. native (blue star grass).
Cortaderia argentea Stapf, vars. *gigantea*, *rosea*, *variegata*,
 S. Amer. Pampas grass, cultivated in N.S.W.
Cyndon incompletus Nees (Stapf), a "blue couch" grass of S
 Af., perhaps indig. in N.S.W.
Danthonia semiannularis R.Br., N.S.W. native (wallaby grass)
racemosa R.Br., N.S.W. native (racemed oat-grass).
Diplachne dubia Scribn., Mexican grass, cultivated Bathurst,
 Hawkesbury.
Eleusine ægyptiaca Pers., N.S.W. native (Egyptian finger grass).
indica Gærtn., N.S.W. native (crab grass).
Leptochloa decipiens R.Br. (Stapf), introduced, interior and
 coast.

NOTES ON THE GRASSES IN TABLE ii.

These twenty species were examined at various seasons, and tested for the presence of cyanogenetic glucoside and enzyme. The results of the various tests are summarised below :—

Methods.—Cyanogenetic compounds were shown to be present in all the species, by plasmolysis of the tissues with vapour of chloroform. (1) Those classified as “very strong” changed colour within one minute, and yielded, in one case, over 0·015% total hydrocyanic acid. (2) Those marked as “strong” gave the colour change within one hour. (3) Those which required to stand 24 hours before any visible change occurred, are described as “faint.”

General Results.—When portions of these grasses are placed in stoppered bottles, with the test paper, but without any reagents, and kept at 37° C. for 24 hours, two species only were found to evolve free hydrocyanic acid, these were *Cynodon incompletus*, and *Diplachne dubia*. The others only gave a positive result after anæsthetising.

Immersing about 10 gm. portions in boiling water does not immediately kill the enzyme; even with 2 minutes' immersion, the grass subsequently liberates hydrocyanic acid when placed in chloroform vapour, but when kept immersed for 2-5 minutes the enzyme is completely destroyed. All the species, when thus treated for 5 minutes, and found to evolve no hydrocyanic acid with chloroform vapour during 48 hours, were then mixed with emulsin, and quickly showed the colour change due to hydrocyanic acid evolution. The compounds were thus shown to be glucosides.

Detailed Results of the Individual Grasses.

Andropogon halepensis.—This grass, which is regarded by Hackel as the original wild species from which the sorghums have sprung, is of very wide distribution, and is now considered indigenous. The reaction of the cyanogenetic glucoside was found to be maximum in January and August, *i.e.*, in the Midsummer growth and the second growth due to the late winter rains. At other times throughout the year, including the flowering period, the grass gave only a “faint” positive reaction.

January	...	+ strong.	August	...	+ strong.
April	...	+ faint.	November	...	+ faint.

No free hydrocyanic acid was evolved from the grass on keeping in a closed bottle for three days.

Andropogon australis has not shown the least trace of hydrocyanic acid at any time of the year.

These two grasses are the only two indigenous sorghums, syn. respectively with *Sorghum halepense* Pers., and *S. plumosum* Beauv.

Andropogon sorghum, vars. *vulgaris*, *saccharatus*.—Grown in experimental plots these grasses were tested in each month, and gave positive reactions from January to December. There was no period in which healthy growing plants were free. In only one plot growth was arrested, and the plants killed, by cold weather in June, and within a few days the tests varied from "strong" to "faint" and nil, the height being 14 inches. Dunstan and Henry found the Egyptian sorghum to lose its glucoside entirely when 14 inches high, while, on the other hand, the sorghum grown here, on the Richmond River, and also that grown in Queensland, showed the presence of glucoside when over 4 feet high.

The glucoside was present in the inflorescence, leaves, stems, and roots. The top leaves were always strongest, and especially the young uncoiled apex-leaves; the reaction diminished with the position of the leaves down the stem, and frequently the lowest leaves gave none. The stems, too, showed a gradual diminution downwards, though frequently they gave uniform reactions. In the roots the strongest reaction was often obtained from the extreme tips.

The leaves also showed a remarkable variation in enzyme, as the following summary of the results, obtained from tests on the leaves of mature plants, will show:—

- i. Leaves anæsthetised, showed strong positive reaction, emulsin added—no evident change produced.
- ii. Leaves anæsthetised, showed faint positive reaction, emulsin added—no evident change produced.
- iii. Leaves anæsthetised, showed faint positive reaction, emulsin added—very strong positive reaction.

iv. Leaves anæsthetised, gave negative result,
 emulsin added—very strong positive reaction.

v. Leaves anæsthetised, showed negative result,
 emulsin added—negative result,
 amygdalin added—strong positive result.

In i. and ii. class of results we have apparently an abundance of enzyme, in iii. a deficiency, and in iv. entire absence. In iv., certain leaves, chiefly the lowest on the stems, contained glucoside alone, the accompanying enzyme having entirely disappeared. In v., certain leaves are shown to contain enzyme only, without glucoside.

The mature plants when cut, and exposed to the air to dry, undergo very little change, with regard to glucoside or enzyme, during the first week. After this, the glucoside is gradually hydrolysed; but while this action is proceeding, the enzyme, too, appears to be slowly destroyed, and so it happens that sometimes it is the glucoside, at other times the enzyme, which first disappears.

Andropogon gryllus.—This indigenous grass never shows more than a trace of glucoside, and that only in the winter; during the hot summer weather it contains none. In autumn, the flowers and also the isolated seeds gave positive reactions.

January	— (young and green).	August	...	+ faint.
April	+ faint.	November	...	+ very faint.

Andropogon intermedius and *A. ischæmum* are two native grasses, which are closely related, and in the summer months give strong reactions for a cyanogenetic glucoside.

		<i>intermed.</i>			<i>ischæm.</i>
January	...	+ strong	+ strong.
April	...	+ faint	+ faint.
August	...	+ faint	+ faint.
November	...	+ strong	+ faint.

Andropogon micranthus.—At no period was more than a trace of hydrocyanic acid detected, even throughout the flowering season, and during the winter months the grass was entirely free.

January	...	+ faint.	August	...	—
April	...	+ faint.	November	...	+ faint.

Bouteloua oligostachya.—This Mexican prairie grass is growing in the neighbourhood of Tenterfield, and specimens from there growing in the Botanic Gardens, were found, at certain seasons, to react strongly for glucoside. In the autumn it entirely disappeared, to return again faintly in the rainy season, and gradually to increase in the Spring, to a maximum at Midsummer.

January ... + very strong. August ... + faint.
April ... - November ... + strong.

Chloris.—Four specimens of this grass are cyanophoric; of these two are native to N.S.Wales, viz., *C. truncata* and *C. ventricosa*, and are widely distributed over the Eastern States.

The exotic species, from which positive results were obtained, are *C. petraea* and *C. polydactyla*, and are cultivated in the Botanic Gardens.

	January.	April.	August.	November.
<i>C. truncata</i> ...	+ strong ...	+ faint ...	- ...	- ...
<i>C. ventricosa</i> ..	+ strong ..	- ...	+ faint ...	+ faint ...
<i>C. petraea</i> ..	+ faint ...	- ...	+ faint	+ very strong
<i>C. polydactyla</i> ..	+ very strong	+ very strong	+ very strong	+ very strong

Samples of the native species were collected by Mr. Breakwell in Narrabri, Wagga, and Coonamble districts, from September to December, and these all gave, during this season, negative results.

Cortaderia argentea.—The three varieties, *gigantea*, *rosea*, *variegata*, growing in the Botanic Gardens, were tested, and also a number of specimens growing elsewhere in Sydney. All gave "strong" reactions in all seasons.

Cynodon incompletus.—This blue couch-grass is recorded only from E. and S. Africa, and in New South Wales from the Upper Hunter River and Forbes. It is still doubtful whether it has been introduced from S. Africa or is indigenous to Australia (Maiden, Agric. Gaz. N.S.Wales, 1912, 295).

Hydrocyanic acid was first detected in this grass in November, 1911, in a patch cultivated in the Botanic Gardens, and which had

been brought by Mr. Maiden from Aberdeen in 1907, from a spot on which cattle had died in November of that year. The cause of the fatality was associated with this grass. Samples were also obtained, through the Chief Inspector of Stock, from Scone and Muswellbrook, and these all gave strong positive reaction at this same season. A second fatality took place at Forbes, where over 100 sheep died on December 9th, 1911. This grass was recognised on the spot, and when tested gave a very strong reaction. A third fatality occurred in the same district in February, 1913; after which some sheep were isolated and fed on this grass alone, when each of them died within half an hour. A sample of this same lot was received for analysis, from the Inspector of Stock at Forbes, and gave the following result:—

	In fresh material.	In grass dried at 100°C.
Free hydrocyanic acid	0·006 %	0·008 %
Combined hydrocyanic acid	0·010 %	0·017 %
Total hydrocyanic acid.....	0·016 %	0·025 %

The free acid was estimated by destroying the enzyme with boiling water, and distilling into standard alkali. The distillate was then titrated with silver nitrate.

The total acid was estimated by previous fermentation of the grass, and then distilling off the volatile acid.

It was calculated from the free acid figure that a sheep of 150 lbs. weight would require, for a lethal dose, to eat about 2 lbs. weight of this grass.

Effect of drying on cut grass.—Grass which gave a very strong reaction for hydrocyanic acid, when exposed openly to the air, showed a gradual diminution of the intensity of reaction during three weeks. At the end of this time the grass reacted only very faintly, and usually in the fourth week gave negative tests. When now, this grass was moistened, and emulsin added, it still gave negative results, but on adding amygdalin instead, a strong positive

result followed. The glucoside alone had disappeared, the enzyme was still active.

A similar result was also obtained with grass which had been air-dried for over three months.

Seasonal variations of *C. incompletus*:—

June	... faint.	December	... very strong.
July	... faint.	January	... very strong.
August	... very faint.	February	... very strong.
September	... faint, increasing.	March	... strong.
October	... strong.	April	... decreasing, faint.
November	... very strong.	May	... faint.

The author desires to express his indebtedness, and thanks to the following gentlemen, for supplies of this grass, at the various seasons:—Chief Inspector Symons, of the Stock Department; Stock Inspectors C. Brooks, of Scone, and W. G. Dowling, of Forbes; Police Inspector Nolan, of Forbes; Mr. J. H. Maiden, F.L.S.

Other Couch-grasses.—*Cynodon dactylon* Pers., the common couch grass of lawns was tested from various parts of the State.

Digitaria didactyla Willd., the Sydney blue couch, is found in certain isolated patches only, such as Hunter's Hill, Vacluse, and Botanic Gardens. These two grasses have always given negative results for hydrocyanic acid, but in a number of instances they showed the presence of an active enzyme capable of hydrolysing amygdalin.

Danthonia semiannularis is generally considered one of the most valuable and nutritious of the native grasses. It gives a faint reaction for cyanogenetic compounds, but towards the end of summer it is parched and dry, and is then quite free, till the autumn rains renew the growth.

January	... + faint.	August	... + faint.
April	... -	November	... + faint.

Samples of this grass were collected by Mr. Breakwell from Narrabri, Wagga, Moree, etc., at the various seasons, and all gave similar results, when tested.

Diplachne dubia, a Mexican grass, cultivated in the Botanic Gardens. This is one of the strongest cyanogenetic grasses tested. It

evolves free hydrocyanic acid continually, and if placed in a stoppered bottle with the test paper, shows an intense reaction in a few minutes. The glucoside, enzyme, and free acid, are present in all parts, and throughout the whole year.

January ... + very strong. August ... + very strong.
 April ... + very strong. November ... + very strong.

Eleusine ægyptiaca and *E. indica*.—These two native grasses are widely distributed, the former in the interior of New South Wales, and the latter in the coastal districts. They are very rich in cyanogenetic glucosides, all parts of the plants giving strong reactions, except in the winter.

				<i>ægypt.</i>	<i>indica.</i>	
January	+	+	strong.
April	+	+	strong.
August	-	-	
November	+	+	strong.

Leptochloa decipiens.—This exotic grass reacts energetically for cyanogenetic glucoside at all times of the year, and is strongest in Autumn and late Spring. The flowers and seeds are also very strong. It is cultivated in the Botanic Gardens and Centennial Park.

Grasses cultivated in the Botanic Gardens.

By the co-operation of the Director of the Gardens, Mr. J. H. Maiden, 152 different species of native and exotic grasses have been tested at four different seasons throughout the year. A number of the results were confirmed by tests on material collected by Mr. E. Breakwell, B.A., B.Sc., Department of Agriculture, in the pastoral districts and at the Government Farms.

All the specimens have been carefully examined by Mr. E. Cheel in the National Herbarium, and considerable time has been occupied in their identification. The species were checked and confirmed by Mr. Maiden, and a number of doubtful ones were referred to Kew. It will be recognised that the value of the results stated is largely dependent on the fact that the botanical names are as correct as it is possible to give them, and for this essential

part of the work much credit is due to my collaborators.

The grasses were tested in a similar manner to the plants recorded in Part i. (These Proc. xxxvii., 1912, 220), viz., (1) grass in vapour of chloroform, for presence of cyanogenetic compounds and free hydrocyanic acid, (2) grass and emulsin, in case of enzyme deficient or absent, and (3) grass and amygdalin, for presence of β enzymes.

These three tests are represented respectively by the three signs in each column.

	Jan.	April.	Aug.	Nov.
<i>Agropyron scabrum</i> Beauv.	- . .	- . .	- . .	- . .
<i>Agrostis alba</i> Linn.	- - -	- . .	- . .	- . .
<i>stolonifera</i> Linn.	- - -	- . .	- . .	- . .
<i>stolonifera</i> Linn., var. <i>gigantea</i>	- - -	- . .	- . .	- . .
<i>verticillata</i> Vill.	- - -	- . .	- . .	- . .
<i>vulgaris</i> With.	- . .	- - -	- . .	- . .
<i>Alopecurus geniculatus</i> Linn.	- . .	- - -	- . .	- . .
<i>Andropogon affinis</i> R.Br.	- - -	- - -	- . .	- . .
<i>annulatus</i> Forst.	- - -	- . .	- . .	- . .
<i>australis</i> Spreng.	- - -	- . .	- . .	- . .
<i>bombycinus</i> R.Br.	- - -	- . .	- . .	- . .
<i>gryllus</i> Trin.	+ . .	+ . .	+ . .	+ . .
<i>halepensis</i> Sibth., var. <i>mutica</i>				
Hack.	+ . .	+ . .	+ . .	+ . .
<i>intermedius</i> R.Br.	+ . .	+ . .	+ . .	+ . .
<i>ischæmum</i> Linn.	+ . .	+ . .	+ . .	+ . .
<i>micranthus</i> Kunth.	+ . .	+ . .	- . .	+ . .
<i>saccharoides</i> Sw., var. <i>barbi-</i>				
<i>nodis</i>	- - -	- - -	- - -	- - -
<i>schenanthus</i> Linn.	- - -	- . .	- . .	- . .
<i>sericeus</i> R.Br.	- - -	- . .	- . .	- . .
<i>Anthoxanthum odoratum</i> Linn.	- . .	+ - -	- . .	- . .
<i>Aristida ramosa</i> R.Br.	- - -	- . .	- - -	- . .
<i>Arundinella nepalensis</i> Trin.	- - -	- . .	- . .	- . .
<i>Asperella hystrix</i> Linn.	- - -	- . .	- . .	- . .
<i>Astrebla triticoides</i> F.v.M.	- - -	- . .	- . .	+ - -
<i>Bouteloua oligostachya</i> Torr.	+ . .	- . .	+ . .	+ . .
<i>Briza minor</i> Linn.	- . .	- . .	- . .	- . .
<i>Bromus erectus</i> Huds.	- - -	- . .	- . .	- . .

	Jan.	April.	Aug.	Nov.
<i>Bromus inermis</i> Leyss	- - -	- . .	- . .	- . .
<i>Kalmii</i> Gray	- - -	- . .	- . .	- . .
<i>madridentis</i> Linn.	- +	- . .	- . .
<i>Pampellianus</i> Scribn.	- - -	- . .	- . .	- . .
<i>racemosus</i> Linn.	- . .	- . .	. +
<i>tectorum</i> Linn.	- - -
<i>unioloides</i> H.B.K.	- - -	- . .	- . .	- . .
<i>Catapodium syrcticum</i> Murb.	- . .	- . .	- . .
<i>Cenchrus australis</i> R.Br.	- . .	- . .	- . .	- . .
<i>Chætum bromoides</i>	- . +	- - -	- . .	- . +
<i>Chloris gayana</i> Kunth.	- - -	- . .	- . .	- . .
<i>petrea</i> Sw.	+ . .	- . .	+ . .	+ . .
<i>polydactyla</i> Sw.	+ . .	+ . .	+ . .	+ . .
<i>submutica</i> H.B.K.	- - -	- . .	- . .	- . .
<i>truncata</i> R.Br.	+ . .	+ . .	- . .	- . .
<i>ventricosa</i> R.Br.	+ . .	- - -	+ . .	+ . .
<i>Coix lachrymi-Jobi</i> Linn.	- - -	- . .	- . .	- . .
<i>Cortaderia argentea</i> , var. <i>rosea</i>	+ . .	+ . .	+ . .	+ . .
var. <i>gigantea</i>	+ . .	+ . .	+ . .	+ . .
var. <i>variegata</i>	+ . .	+ . .	+ . .	+ . .
<i>Corynephorus canescens</i> Beauv.	- - +	- - +	- . .	- . .
<i>Cynodon dactylon</i> Pers.	- - +	- . +	- - -	- . .
<i>incompletus</i> Nees	+ . .	+ . .	+ . .	+ . .
<i>Dactylis glomerata</i> Linn.	- . .	- . .	- . .
<i>Danthonia semiannularis</i> R.Br.	+ . .	- . .	+ . .	+ . .
<i>racemosa</i> R.Br.	+ . .	- . .	- . .	- . .
<i>Dichelachne crinita</i> Hook.	- - -	- . .	- . .	- . .
<i>Digitaria didactyla</i> Willd.
<i>tenuiflora</i> Beauv.	- . .	- . .	- . .
<i>Diplachne dubia</i> Scribn.	+ . .	+ . .	+ . .	+ . .
<i>Echinopogon ovatus</i> Palis.
<i>Ehrharta calycina</i> Sw., var. <i>versicolor</i>	- - -	- - -	- - -	- - -
<i>Eleusine ægyptiaca</i> Pers.	+ . .	+ . .	- . .	+ . .
<i>indica</i> Gært.	+ . .	+ . .	- . .	+ . .
<i>Elymus arenareus</i> Linn.	- - -	- - -	- - -	- - -
<i>robustus</i> Scribn.	- - -	- - -	- - -	- - -
<i>virginicus</i> Linn.	- - -	- - -	- - -	- - -
<i>Eragrostis Brownii</i> Nees	- - -	- . .	- - -	- . .
<i>curvula</i> Nees.	- - -	- . .	- - -	- . .
<i>diandra</i> Steud.	- . .	- - -	- - -	- . .

	Jan.	April.	Aug.	Nov.
<i>Eragrostis leptostachya</i> Steud	- - -	- . .	- . .	- . .
<i>major</i>	- . .	- . .	- . .	- . .
<i>pilosa</i> Beauv.	- - +	- - +	- . .	- . .
<i>plana</i> Nees	- - -	- - -	- - -	- - -
<i>purshii</i> Schrad.	- - +	. . .	- . +	- . -
<i>Erianthus ravennae</i> Beauv.	- - -	- . .	- . .	- . -
<i>Festuca bromoides</i> Linn.	- - -	. . .
<i>duriuscula</i> Linn.	- - -	. . .	- . .	- . -
<i>elatior</i> Linn.	- - -	. . .	- . .	- . -
<i>elatior</i> Linn., subsp. <i>arundinacea</i>	- - -	. . .	- . .	- . -
<i>gigantea</i> Vill.	- - -	. . .	- . .	- . -
<i>Hookeriana</i> F.v.M.	- - -	. . .	- . .	- . .
<i>ovina</i> Linn.	- - -	. . .	- . .	- . -
<i>ovina</i> , var. <i>tenuifolia</i> , Sibth. . .	- - -	. . .	- . .	- . -
<i>rubra</i> Linn.	- - -	. . .	- . .	- . -
<i>Glyceria Fordeana</i> F.v.M.	- - -	-
<i>Hæmarthria compressa</i> R.Br.	- . +	. . .	- - +	- . .
<i>Isachne australis</i> R.Br.	- . -	- . .	- . .	- . -
<i>Lagurus ovatus</i> Linn.	- . +	- . -	- . +
<i>Leptochloa decipiens</i> R.Br. (Stapf)	+ . .	+ . .	+ . .	+ . .
<i>Lolium multiflorum</i> Lam.	- - -	- . .	- . -
<i>perenne</i> Linn.	- . .	- . .	- . -
<i>temulentum</i> Linn.	- . -	- . .	- . .	- . -
<i>Microlæna stipoides</i> R.Br.	- - -	. . .	- . .	- . -
<i>Miscanthus sinensis</i> , var. <i>zebrina</i> . .	- . .	- . .	- . .	- . -
<i>Oplismenus Burmanni</i> Beauv., var.				
<i>variegatus</i>	- . .	- . -	. . .	- . -
<i>Oryzopsis miliacum</i> Benth.	- - -	- . .	- . .	- . -
<i>Panicum bicolor</i> R.Br.	- - -	- - -	- - -	- . -
<i>bulbosum</i> H.B.K.	- - +	- . -	. . +	- . -
<i>colonum</i> Linn.	- - +	- . -
<i>decompositum</i> R.Br.	- - -	. . .	- . .	- . -
<i>divaricatissimum</i> R.Br., var.				
<i>normale</i> Benth.	- . +	- . .	- . .	- . +
<i>flavidum</i> Retz.	- - -	- . .	- . .	- . -
<i>flavidum</i> , var. <i>tenuior</i> Retz. . .	- . -	- . .	- . .	- . -
<i>gracile</i> R.Br.	- - -	- . .	- . .	- . -
<i>leucophæum</i> H.B.K.	- - +	- +	- . .	- . .
<i>marginatum</i> R.Br.	- . -	- - -	- . .	- . -
<i>miliare</i> Lam.	- . .	- . -

	Jan.	April.	Aug.	Nov.
<i>Panicum parviflorum</i> R.Br.	- - -	- . .	- . .	- . .
<i>plicatum</i>	- . .	- . .	- - -	- . .
<i>sanguinale</i> Linn.	- - -	- . .	- . .	- . .
<i>strictum</i> R.Br.	- . .	- . .	- + -	- . .
<i>teneriffie</i> R.Br.	- . .	- . .	- . .	- . .
<i>Paspalum dilatatum</i> Poir.	- - -	- . .	- . .	- . .
<i>distichum</i> Linn.	- - -	- . .	- . .	- . .
<i>laeve</i> Michx.	- - -	- . .	- . .	- . .
<i>paniculatum</i> Linn.	- - -	- . .	- . .	- . .
<i>platycaule</i> Poir.	- - -	- . .	- + -	- + -
<i>scrobiculatum</i> Linn.	- . .	- . .	- + -	- . .
<i>stoloniferum</i> Desv.	- - -	- . .	- . .	- . .
<i>undulatum</i> Poir.	- . .	- - -	- . .	- . .
<i>virgatum</i> Linn.	- - -	- . .	- . .	- . .
<i>Pennisetum compressum</i> R.Br.	- - -	- . .	- . .	- . .
<i>latifolium</i> Spreng.	- + -	- . .	- . .	- . .
<i>longistylum</i> Hochst.	- - +	- . .	- . .	- . .
<i>macrorum</i> Trin.	- - -	- . .	- . .	- . .
<i>orientale</i> Rich., var. <i>triflorum</i>	- - -	- . .	- . .	- . .
<i>Phalaris bulbosa</i> Linn.	- . .	- . .	- . .	- . .
<i>cærulescens</i> Desf.	- . .	- . .	- . .	- . .
<i>minor</i> Retz....	- . .	- . .	- . .	- . .
<i>Poa annua</i> Linn.	- . .	- . .	- . .	- . .
<i>cæspitosa</i> Forst.	- - -	- . .	- . .	- . .
<i>compressa</i> Linn.	- - -	- . .	- . .	- . .
<i>nemoralis</i> Linn.	- - +	- + -	- + -	- . .
<i>pratensis</i> Linn.	- - -	- . .	- . .	- . .
<i>Pollinia fulva</i> Benth.	- - -	- . .	- . .	- . .
<i>Saccharum officinarum</i> Linn.	- - -	- . .	- . .	- . .
<i>sara</i>	- - -	- . .	- . .	- . .
<i>Secale dalmaticum</i> Vis.	- - -	- . .	- . .	- . .
<i>Setaria imberbis</i> Roem. & Schult..	- . .	- . .	- . .	- . .
<i>Spinifex hirsutus</i> Labill.	- . .	- . .	- . .	- . .
<i>Sporobolus diander</i> Beauv.	- - -	- . .	- . .	- . .
<i>indicus</i> R.Br.	- - -	- . .	- . .	- . .
<i>virginicus</i> Kunth.	- . .	- - -	- - +	- . .
<i>Wrightiana</i>	- . .	- . .	- . .	- . .
<i>Stipa elegantissima</i> Labill.	- . .	- . .	- + -	- . .
<i>pubescens</i> R.Br.	- . .	- . .	- . .	- . .
<i>tenuissima</i> Trin.	- - -	- . .	- . .	- + -

	Jan.	April.	Aug.	Nov.
<i>Stipa verticillata</i> Trin.	- - -	- . .	- - +	- . -
<i>Themeda avenacea</i>	- - -	- . .	- . -	- . .
<i>Forskali</i> Hack.	- - -	- . .	- . .	- . .
<i>gigantea</i> Hack.	- - -	- - -	- . -	- . -
<i>Triodia albescens</i> Munro	- - -	- . .	- . .	- . -
<i>Trypsacum dactyloides</i> Linn.	- - -	- . .	- . .	- . -
<i>Uniola latifolia</i> Michx.	- - +	- . -	- . .	- . -
<i>Zoysia pungens</i> Willd.	- - -	- . -	- - +	- . -

The author desires to express his indebtedness to Professor Anderson Stuart for laboratory facilities afforded for this investigation.